# The Cholesterol-Lowering Action of Plant Stanol Esters

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**ABSTRACT Plant sterols and stanols derived from wood pulp and vegetable oils lower total and LDL cholesterol by inhibiting cholesterol absorption from the intestine in humans. Plant stanols are virtually unabsorbable, which makes them more ideal hypocholesterolemic agents than plant sterols. The esterification of plant stanols has allowed their incorporation into various foods such as margarine without changing the taste and texture of those foods. Plant stanol esters at a level of 2–3 g/d have been shown to reduce LDL cholesterol by 10–15% without side effects. Plant stanol esters appear to be a helpful dietary adjunct to a prudent diet to lower cholesterol. J. Nutr. 129: 2109–2112, 1999.**

KEY WORDS: • sitostanol • sitosterol • cholesterol absorption • cholesterol

The cholesterol-lowering effect of dietary plant sterols (phytosterols) has been studied since the 1950s and is well known (Lees et al. 1977). Earlier studies showed that large amounts of sitosterol (≥10 g/d) lowered serum cholesterol levels by 10–20%. The high dosage and the chalky taste of sitosterol limited its use, especially with the advent of the more powerful, well-tolerated, lipid-lowering 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Grundy and Mok (1976) subsequently demonstrated that 3 g/d of sitosterol was sufficient to lower serum cholesterol levels. They suggested that plant sterols could be considered a form of dietary treatment rather than a drug to lower cholesterol because plant sterols are naturally present in plant-based foods.

The differences in the various plant sterols became apparent when saturated derivatives of plant sterols, called plant stanols, were shown to reduce serum cholesterol at low doses. New techniques allowed the incorporation of plant stanols into food forms without affecting the texture and taste. In 1995, the Finnish introduced plant stanol esters (PSE)[2] in margarine, as dietary adjuncts to lower cholesterol (Cater and Grundy 1998).

***Plant Sterols and Stanols Structure and Function.*** Plant sterols are C-28 or C-29 sterols, differing from cholesterol (C-27) by the presence of an extra methyl or ethyl group on the cholesterol side chain (**Fig. 1**). Cholesterol is an essential component of cell membranes in higher species. Plant sterols play an analogous role in plants; their content is highest in edible oils, seeds and nuts (Weihrauch and Gardner 1978).

The major dietary sterols are sitosterol (C-29), campesterol (C-28) and stigmasterol (C-29). These represent <50% of the total intake of sterols in the Western diet; the remainder is cholesterol (Subbiah 1971). The most common dietary plant stanol, sitostanol, is a saturated derivative of sitosterol. It occurs naturally in wood pulp, tall oil and, in lesser amounts, in soybean oil. The Western daily diet contains 100–300 mg plant sterols and 20–50 mg plant stanols (Czubayko et al. 1991).

***Absorption and metabolism.*** The addition of a methyl or ethyl group on the side chain of cholesterol results in poor intestinal absorption of plant sterols in humans (Subbiah 1973). Thus, only 1.5–5% of sitosterol is absorbed when typical amounts of sterols are consumed (240–320 mg) (Kritchevsky 1997). Cholesterol absorption is much more efficient, with between 20 and 80% of dietary cholesterol absorbed. Differential absorption rates among plant sterols are related to the length of the side chain. The longer the side chain of the sterol, the less is absorbed because of its increased hydrophobicity (Heinemann et al. 1993). Serum levels of sitosterol are 0.3–1.7 mg/dL (Glueck et al. 1991, Salen et al. 1970), given a dietary intake of 160–360 mg/d of plant sterols. This wide range in a normal population suggests considerable individual variability in the handling of various plant sterols. Consumption of 3.24 g/d of plant sterols has been shown to increase serum sitosterol and campesterol levels by an average of 40 and 70%, respectively (Westrate and Meijer 1998). Because dietary plant sterols can initiate the development of atherosclerosis (Bhattacharyya and Connor 1974) and may increase the risk of premature coronary heart disease (CHD) in hypercholesterolemic patients (Glueck et al. 1991), the lowest serum levels of sterols are desirable. Thus, Lees and Lees (1976) suggested that plant sterol preparations that contain more absorbable sterols such as campesterol should not be recommended for therapeutic use.

Hydrogenation of plant sterols to the corresponding stanols renders them virtually unabsorbable (Subbiah 1973). Absorption of sitostanol has been estimated to be between 0 and 3%, and serum levels are practically undetectable (Gylling et al. 1999, Westrate and Meijer 1998). The absorption of the other major stanol, campestanol, is also very low, in contrast to its unsaturated counterpart, campesterol (Xu et al. 1999).

***Mechanism of action.*** Plant sterols interfere with the uptake of both dietary and biliary cholesterol from the intestinal tract in humans (Heinemann et al. 1991). The reason for this is not fully understood; however, plant sterols appear to decrease the solubility of cholesterol in the oil and micellar phases, thus displacing cholesterol from bile salt micelles and interfering with its absorption (Ikeda and Sugano 1998). In humans, intestinal infusion of sitostanol was more efficient in reducing cholesterol absorption than infusion of sitosterol (−85% and −50%, respectively) (Heinemann et al. 1991). In addition, Becker et al. (1993) showed that 1.5 g/d of sitostanol increased fecal secretion of neutral and acid steroids more effectively (88%) than did 6 g/d of sitosterol (45%).

It has been proposed that sitostanol, which is relatively unabsorbable compared with sitosterol, remains in the intestinal lumen where it can interfere continuously and more efficiently with micellar solubility of cholesterol (Ikeda and Sugano 1998). Another important determinant of the effectiveness of these compounds is how well they mix with intestinal contents for proper physical presentation to the gut. When compared with the unesterified stanols, the fatty acid esters of stanols seem to mix more easily with the oil phase of the intestinal contents to interfere with cholesterol absorption and decrease plasma cholesterol concentrations (Vanhanen et al. 1993).

In addition to reducing absorption of cholesterol, plant stanols inhibit absorption of other plant sterols (Gylling et al. 1999). In

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[2] Abbreviations used: apo, apoprotein; CHD, coronary heart disease; HDL-C, HDL cholesterol; IDL-C, intermediate density lipoprotein cholesterol; NCEP, National Cholesterol Education Program; PSE, plant stanol esters; TC, total cholesterol; VLDL-C, VLDL cholesterol.

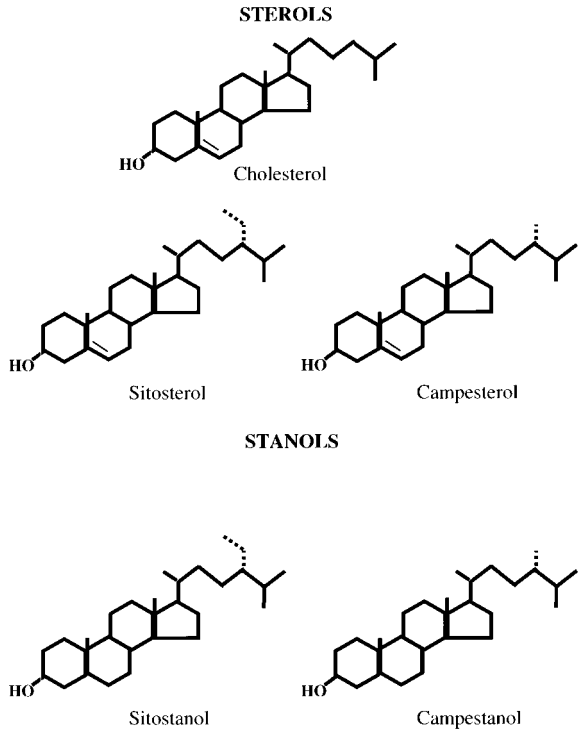


**FIGURE 1** Structures of sterols, including cholesterol and the plant sterols, sitosterol and campesterol, and the corresponding stanols, sitostanol and campestanol.

humans, the inhibition of intestinal cholesterol absorption is accompanied by a compensatory increase in cholesterol synthesis, as reflected in the increase in the serum cholesterol precursors, lathosterol and desmosterol. However, the net effect is still reduction in serum cholesterol.

***Hypocholesterolemic Effect of Plant Sterols and Stanols.*** The lower absorbability of sitostanol is thought to be responsible for its greater hypocholesterolemic effect compared with sitosterol and campesterol (Jones et al. 1997) In addition, Vanhanen et al. (1994) showed that the ester form of sitostanol is more efficient than the crystalline form. Indeed, in the only study that showed no significant cholesterol-lowering effect with 3g/d of sitostanol, sitostanol was given in a crystalline form that may have limited its efficacy (Denke 1995).

Miettinen and co-workers (1995) esterified sitostanol into rapeseed oil fatty acids, thus allowing them to put a large amount into margarine without affecting its texture or taste. **Table 1** summarizes the clinical studies with PSE in various populations. The majority of the early studies on stanol ester–fortified foods were done in Finnish population studies. PSE dosages have ranged in various studies from 0.8 to 3.8 g/d. The data suggest that at least 1 g/d of stanol esters must be consumed to offer a good clinical response. In general, with consumption of 2–3 g/d of PSE, serum LDL cholesterol (LDL-C) levels were lowered between 10 and 15%. It is difficult to compare these studies in terms of dose response because of differences in background diet, baseline lipid levels and duration of treatment. It has been suggested that consumption of >3 g/d of PSE may not further decrease the cholesterol-lowering effect (Mensink and Plat 1998). The narrow range of dose responsiveness may be due to the compensatory increase in cholesterol synthesis that can be observed after consumption of higher doses of plant sterols and stanols. Indeed, Vanhanen et al. (1994) calculated that intake of 2 g/d of sitostanol esters increased cholesterol synthesis by 2 mg/(d · kg body weight), although there was still a net reduction in serum cholesterol. This was not seen with 0.8 g/d of sitostanol esters.

Only a few studies have evaluated directly the dose-response relationship of PSE. Miettinen et al. (1995) compared 1.8 and 2.6 g/d of PSE and showed a significantly greater cholesterol-lowering effect of the higher dose on total cholesterol (TC) (10.2% with 2.6 g/d vs. 9.3% with 1.8 g/d), although the difference was small. Nguyen et al. (1998) showed a trend for greater efficacy of 3 g/d of PSE ($n$ = 20) compared with 2.1 g/d of PSE ($n$ = 19). There was a reduction in TC of 9.4 and 5.6%, respectively, after 8 wk, compared with placebo

**TABLE 1**

*Summary of clinical studies with plant stanol esters (PSE)*

| Study | Subjects[1] | PSE intake | Baseline cholesterol | Dietary cholesterol | Duration of use | TC reduction[2] | LDL-C reduction[2] |
|---|---|---|---|---|---|---|---|
| | *n* | *g/d* | *mmol/L* | *mg/d* | *wk* | % | |
| Vanhanen et al 1994 | 7 (8) | 0.8 | 5.5 | 265 | 6 | 5 | 7 |
| Miettinen and Vanhanen 1994 | 7 (8) | 0.8 | 5.5 | 294 | 9 | 5[3] | 8[3] |
| Miettinen et al. 1995 | 51 (51) | 1.8 | 6.0 | 308 | 52 | 9[3] | 11[3] |
| Vanhanen et al. 1994 | 7 (8) | 2.0 | 5.5 | 265 | 6 | 9[3] | 16[3] |
| Niinikoski et al. 1997 | 12 (12) | 2.2 | 5.0 | NR | 5 | 10[3] | 13[3] |
| Hallikainen and Uusitupa 1999 | 20 (17) | 2.2 | 6.1 | 141 | 8 | 8[3] | 9[6] |
| Hallikainen and Uusitupa 1999 | 18 (17) | 2.4 | 6.6 | 162 | 8 | 11[3] | 14[6] |
| Westrate and Meijer 1998 | 100 (100) | 2.5 | 5.2 | 243 | 3.5 | 7[3] | 13[6] |
| Miettinen et al. 1995 | 51 (51) | 2.6 | 6.1 | 340 | 52 | 10[3] | 14[6] |
| Gylling and Miettinen 1994 | 11[4] (11) | 3.0 | 6.0 | 357 | 6 | 5[6] | 9[6] |
| Gylling and Miettinen 1996 | 8[4] (8) | 3.0 | 6.6 | 233 | 7 | 11[6] | 14[6] |
| Gylling et al. 1997 | 11[5] (11) | 3.0 | 6.0 | 207 | 7 | 8[6] | 15[6] |
| Gylling et al. 1995 | 15[6] (15) | 3.0 | 7.7 | 114 | 6 | 11[6] | 15[6] |
| Vanhanen, et al. 1993 | 34 (33) | 3.4 | 5.9 | 270 | 6 | 8[6] | 10[6] |
| Plat et al. 1998 | 34 (42) | 3.7 | 5.0 | 230 | 8 | 8[6] | 12[6] |
| Plat et al. 1998 | 36 (42) | 3.8 | 5.0 | 247 | 8 | 8[6] | 13[6] |

[1] Adult men and women in PSE group unless indicated; *n* for control group in parenthesis.
[2] Difference between control and PSE group in % total cholesterol or LDL cholesterol change from baseline to end of study.
[3] $P < 0.05$ vs. control.
[4] Type 2 diabetics.
[5] Postmenopausal women.
[6] Familial hypercholesterolemia children.

More studies are required to test the optimal intake of PSE to lower serum cholesterol, particularly above 3.7 g/d.

Most studies comparing plant stanols with plant sterols have shown the greater potency of plant stanols in lowering serum cholesterol. One notable exception is a crossover, randomized study by Westrate and Meijer (1998); that study showed that soybean sterol ester margarine lowered LDL-C by 13%, as much as the plant stanol ester margarine, Benecol. However, the daily intake of plant sterols in the soybean preparation (3.24 g/d) was greater than that in Benecol (2.74 g/d). The fatty acid composition of the two differed as well. The soybean sterol ester margarine was lower in saturated fatty acids and contained more linoleic acid than does Benecol. It is well established that linoleic acid lowers blood cholesterol compared with other more saturated fatty acids (Mensink and Katan 1992). This difference in the fatty acid profiles of the spreads in that study may have underestimated the hypocholesterolemic effect of Benecol compared with the soybean sterol ester margarine by ~2% in TC and LDL-C reduction. Nevertheless, the cholesterol-lowering efficacy of plant stanols and plant sterols was quite comparable in that study. This may be due in part to the fact that the plant sterols were esterified. More studies are required to determine whether esterification can render plant sterols as effective as esterified plant stanols in lowering cholesterol.

***PSE effect on other lipids.*** In contrast to lowering TC and LDL-C, PSE did not exert a significant effect on HDL-C and TG in most studies (Jones et al. 1997). In a study of hypercholesterolemic type-2 diabetic patients, 3 g/d of PSE decreased VLDL cholesterol (VLDL-C) by 12%, intermediate density lipoprotein cholesterol (IDL-C) by 11%, whereas HDL-C increased by 11% ($P < 0.05$) (Gylling and Miettinen 1994). The reason for this is not entirely understood; however, it is thought to result from an increased removal of remnant particles by up-regulation of LDL receptor activity.

***Determinants of response to stanol esters.*** Which subjects would be most responsive to PSE? Several variables have been examined as potential predictors of response to PSE. Vanhanen et al. (1993) showed that the decrease in LDL-C is greater in the apoprotein (apo) E-4 homozygote group than that in the apo E-3 homozygote group. This can be explained presumably by the fact that baseline cholesterol absorption is higher in subjects with the E-4 allele. On the other hand, the LDL-C reduction was shown to be similar for different apo E genotypes in 70 subjects consuming 3.7–3.8 g/d of PSE (Plat et al. 1998).

PSE appear to be most effective in lowering cholesterol in subjects with a high ability to absorb cholesterol and a lower cholesterol synthesis rate (Gylling et al. 1999). The degree of reduction in LDL-C by PSE has been shown to correlate significantly with the magnitude of the PSE effect on the efficiency of cholesterol absorption (Miettinen et al. 1995). In addition, subjects with high baseline cholesterol absorption, as reflected by high serum levels of plant sterols, had a greater cholesterol-lowering response to PSE. On the other hand, a high cholesterol synthesis rate at baseline predicted a smaller decrease in cholesterol absorption with consumption of PSE. These types of patients could potentially be identified by measuring serum levels of precursor sterols in cholesterol synthesis and serum levels of plant sterols such as campesterol and sitosterol. However, this is currently not a practical approach in a clinical setting.

Early studies of plant sterols in humans suggested that the relatively high dietary cholesterol concentrations (mean, 282–340 mg/d) may have contributed to the favorable results (Mattson et al. 1982). It has been suggested that the weak response in one study with crystalline sitostanol may have been explained by a low dietary cholesterol intake (<200 mg/dL) (Denke 1995). Most studies with PSE have been conducted in subjects after consumption of a diet higher in fat and cholesterol than the average American diet. In a landmark study with plant stanol ester margarine (Miettinen et al. 1995), the average saturated fat intake was 14% and cholesterol intake was 340 mg/d. The question arises whether plant stanols can be as effective during a period of more restricted dietary saturated fat and cholesterol intake. Hallikainen and Uusitupa (1999) recently studied subjects consuming a diet that followed closely the National Cholesterol Education Program (NCEP) step 2 diet, in which the mean total fat intake was 26%, saturated fat intake was 6.9% and cholesterol intake was 146 mg/d. They showed that >2 g/d of PSE lowered mean total and LDL-C by up to 10.6 and 13.7% respectively, compared with a control group.

Thus, PSE can reduce cholesterol even in the presence of a low dietary cholesterol intake (<200 mg/d), supporting the notion that PSE can interfere with the absorption of both dietary cholesterol and biliary cholesterol.

***Hypocholesterolemic effect of PSE in combination with lipid-lowering drugs.*** PSE appear to have a synergistic effect in lowering cholesterol when combined with lipid-lowering agents that act at other steps of lipid metabolism. The combination of 3 g/d of PSE with 10–20 mg of simvastatin reduced TC and LDL-C by an additional 11 and 16% respectively (Gylling et al. 1997). Of clinical significance, recent data suggest that subjects who had low cholesterol synthesis and high cholesterol absorption capacity at baseline were more likely to have a recurrent CHD event despite simvastatin treatment (Miettinen et al. 1996). It is tempting to speculate that combination treatment with PSE in those patients would improve the response and help prevent the recurrence of coronary events. However, the only pertinent atherosclerosis prevention data with plant stanols exist in animal models at present (Ikeda and Sugano 1998).

***Side Effects of Plant Stanols and Sterols.*** No significant side effects, including gastrointestinal side effects, have been observed with consumption of plant stanol esters. Consumption of stanol esters by humans in a 12-mo study showed excellent compliance without obvious side effect or weight gain (Miettinen et al. 1995). In addition, blood variables remained within normal range. Hypercholesterolemic children given sitostanol for 7 mo were also free from ill effects (Becker et al. 1993).

At high levels of intake, the potential exists for an estrogenic effect of plant sterols but not plant stanols. When sitosterol was injected into male rats, testicular weight and sperm concentration decreased (Malini and Vanithakumari 1991). An increase in the uterine weight of female rats (Malini and Vanithakumari 1993) and an increase in basal luteinizing hormone secretion in immature male and female rabbits were also observed (Register et al. 1995).

The clinical significance of these findings in humans is unknown. In contrast to sitosterol, sitostanol has not been found to have an estrogenic effect (Mellanen et al. 1996). Furthermore, in postmenopausal women who consumed 3 g/d of PSE margarine for 14 wk, there was no significant change in estradiol levels (Gylling and Miettinen 1998).

***Effect on lipophilic dietary compounds.*** Studies exist that have examined the effect of plant sterol esters and stanol esters on the absorption of lipophilic compounds such as β-carotene and fat-soluble vitamins. Both plant sterols and stanols may lower serum concentrations of carotene (Gylling et al. 1996, Westrate and Meijer 1998); however, this finding is not universal. Hallikainen and Uusitupa (1999) observed no significant change in lipid standardized β-carotene levels with PSE consumed as part of a NCEP step 2 diet. In other studies that have shown a significant effect on β-carotene levels from PSE consumption, the subjects were consuming a higher fat intake. This suggests that increasing the intake of carotenoid-rich foods such as fruits and vegetables as part of a low fat diet can negate the decrease in β-carotene levels that had been observed with PSE.

More significantly, PSE have not been shown to have a significant effect on the fat-soluble vitamin, retinol, for which β-carotene is the precursor. Nor was there an effect on 25-OH vitamin D levels. α-Tocopherol levels were decreased with consumption of PSE; however, the lipid standardized α-tocopherol levels were unchanged. No effect of consumption of PSE on vitamin K–dependent hemostasis in anticoagulated patients has been seen (Nguyen and Dale 1999). Finally, no drug interaction with PSE has been observed to date.

## LITERATURE CITED

Becker, M., Staab, D. & von Bergmann, K. (1993) Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. J. Pediatr. 122: 292–296.

Bhattacharyya, A. K. & Connor, W. E. (1974) β-Sitosterolemia and xanthomatosis: a newly described lipid storage disease in two sisters. J. Clin. Investig. 53: 1033–1043.

Cater, N. B. & Grundy, S. M. (1998) Lowering serum cholesterol with plant sterols and stanols. Historical perspectives. In: New Developments in the

Dietary Management of High Cholesterol (Nguyen, T. T., ed.), pp. 6–14. Health Care Information Programs, McGraw-Hill Health Care Publications Group (Postgraduate Medicine Special Report), Minneapolis, MN.
Czubayko, F., Beumers, B., Lammsfuss, S., Lutjohann, D. & von Bergmann, K. (1991) A simplified micro-method for quantification of fecal excretion of neutral and acidic sterols for outpatient studies in humans. J. Lipid Res. 32: 1861–1867.
Denke, M. A. (1995) Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am. J. Clin. Nutr. 61: 392–396.
Glueck, C. J., Speirs, J., Tracy, T., Streicher, P., Illig, E. & Vandegrift, J. (1991) Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. Metab. Clin. Exp. 40: 842–848.
Grundy, S. M. & Mok, H. Y. (1976) Effect of low dose phytosterols on cholesterol absorption in man. In: Lipoprotein Metabolism (Greten, H., ed.), pp. 112–118, Springer-Verlag, Berlin, Germany.
Gylling, H. & Miettinen, T. A. (1994) Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolaemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia 37: 773–780.
Gylling, H. & Miettinen, T. A. (1995) The effect of cholesterol absorption inhibition on low density lipoprotein cholesterol level. Atherosclerosis 117: 305–308.
Gylling, H. & Miettinen, T. A. (1996) Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin-dependent diabetic men. J. Lipid Res. 37: 1776–1785.
Gylling, H. & Miettinen, T. A. (1998) Efficacy of plant stanol ester in lowering cholesterol in postmenopausal women and patients with diabetes. In: New Developments in the Dietary Management of High Cholesterol (Nguyen, T. T., ed.), pp 39–43, Health Care Information Programs, McGraw-Hill Health Care Publications Group (Postgraduate Medicine Special Report), Minneapolis, MN.
Gylling, H., Puska, P., Vartianen, E. & Miettinen, T. A. (1996) Serum retinol, $\alpha$-tocopherol, carotenes and lipid peroxide production during serum cholesterol lowering by sitostanol ester margarine in a mildly hypercholesterolemic population. Circulation 94: I-578 (abs.).
Gylling, H., Puska, P, Vartiainen, E. & Miettinen, T. A. (1999) Serum sterols during stanol ester feeding in a mildly hypercholesterolemic population. J. Lipid Res. 40: 593–600.
Gylling, H., Radhakrishnan, R. & Miettinen, T. A. (1997) Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine: women and dietary sitostanol. Circulation 96: 4226–4231.
Gylling, H., Siimes, M. A. & Miettinen, T. A. (1995) Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. J. Lipid Res. 36: 1807–1812.
Hallikainen, M. A. & Uusitupa, M. I. (1999) Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am. J. Clin. Nutr. 69: 403–410.
Heinemann, T., Axtmann, G. & von Bergmann, K. (1993) Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur. J. Clin. Investig. 23: 827–831.
Heinemann, T., Kullak-Ublick, G. A., Pietruck, B. & von Bergmann, K. (1991) Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol. Eur. J. Clin. Pharmacol. 40 (suppl 1): S59–S63.
Ikeda, I. & Sugano, M. (1998) Inhibition of cholesterol absorption by plant sterols for mass intervention. Curr. Opin. Lipidol. 9: 527–531.
Jones, P.J.H., MacDougall, D. E, Ntanios, F. & Vanstone, C. A. (1997) Dietary phytosterols as cholesterol-lowering agents in humans. Can. J. Physiol. Pharmacol. 75: 217–227.
Kritchevsky, D. (1997) Phytosterols. In: Dietary Fiber in Health and Disease (Kritchevsky, D. & Bonfield, C., eds.), pp. 235–243, Plenum Press, New York, NY.
Law, M. R., Wald, N. J., Wu, T., Hackshaw, A. & Bailey, A. (1994) Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study. Br. Med. J. 308: 363–366.
Lees, R. S. & Lees, A. M. (1976) Effects of sitosterol on plasma lipid and lipoprotein concentration. In: Lipoprotein Metabolism (Greten, H., ed.) pp. 119–130, Springer-Verlag, Berlin, Germany.
Lees, A. M., Mok, H. Y., Lees, R. S., McCluskey, M. A. & Grundy, S. M. (1977) Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28: 325–338.
Malini, T. & Vanithakumari, G. (1991) Antifertility effects of $\beta$-sitosterol in male albino rats. J. Ethnopharmacol. 35: 149–153.
Malini, T. & Vanithakumari, G. (1993) Effect of $\beta$-sitosterol on uterine biochemistry: a comparative study with estradiol and progesterone. Biochem. Mol. Biol. Int. 31: 659–668.
Mattson, F. H., Grundy, S. M. & Crouse, J. R. (1982) Optimizing the effect of plant sterols on cholesterol absorption in man. Am. J. Clin. Nutr. 35: 697–700.
Mellanen, P., Petanen, T., Lehtimaki, J., Makela, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A. & Santti, R. (1996) Wood-derived estrogens: studies in vitro with breast cancer cell lines and in vivo in trout. Toxicol. Appl. Pharmacol. 136: 381–388.
Mensink, R. P. & Katan, M. B. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. Arterioscler. Thromb. 12: 911–919.
Mensink, R. P. & Plat, J. (1998) Efficacy of dietary plant stanols. In: New Developments in the Dietary Management of High Cholesterol (Nguyen, T. T., ed.), pp. 27–31, Health Care Information Programs, McGraw-Hill Health Care Publications Group (Postgraduate Medicine Special Report), Minneapolis, MN.
Miettinen, T. A., Puska, P., Gylling, H., Vanhanen, H. & Vartiainen, E. (1995) Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N. Engl. J. Med. 333: 1308–1312.
Miettinen, T. A., Strandberg, T., Vanhanen, H. & Gylling, H. for the Scandinavian Simvastatin Survival Study Group (1996) Non-cholesterol serum sterols in Scandinavian Simvastatin Survival Study. In: Drugs Affecting Lipid Metabolism, Vol. 10 (Gotto, A. M., Jr., Paoletti, R., Smith, L. C., Catapano, A. L. & Jackson, A. S., eds.), pp. 473–476. Kluwer Academic Publishers, Medical Science Symposium Series, Dordrecht, The Netherlands.
Miettinen, T. A. & Vanhanen, H. (1994) Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotype. Atherosclerosis 105: 217–226.
Nguyen, T., Croghan, I. & Dale, L. C. (1998) Effects of three different phytostanol ester enriched spreads on serum lipoproteins in a mildly hypercholesterolemic US population. Abstract, IV Cardiovascular Disease Prevention Conference, London, England.
Nguyen, T. T. & Dale, L. C. (1999) Plant stanol esters and vitamin K. Mayo Clin. Proc. 74: 642–643.
Niinikoski, H., Viikari, J. & Palmu, T. (1977) Cholesterol-lowering effect and sensory properties of sitostanol ester margarine in normocholesterolemic adults. Scand. J. Nutr. 41: 9–12.
Plat, J., Mensink, R. P. & Hornstra, G. (1998) Effects of a vegetable oil based or pine wood based phytostanol ester mixtures on serum lipoproteins in normocholesterolemic subjects. Abstract, XII International Symposium on Drugs Affecting Lipid Metabolism, Florence, Italy.
Register, B., Bethel, M. A., Thompson, N., Walmer, D., Blohm, P., Ayyash, L. & Hughes, C., Jr. (1995) The effect of neonatal exposure to diethylstilbestrol, coumesterol, and $\beta$-sitosterol on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Proc. Soc. Exp. Biol. Med 208: 72–77.
Salen, G., Ahrens, E.H.J. & Grundy, S. M. (1970) Metabolism of $\beta$-sitosterol in man. J. Clin. Investig. 49: 952–967.
Subbiah, M.T.R. (1971) Significance of dietary plant sterols in man and experimental animals. Mayo Clin. Proc. 46: 549–559.
Subbiah, M. T. (1973) Dietary plant sterols: current status in human and animal sterol metabolism. Am. J. Clin. Nutr. 26: 219–225.
Vanhanen, H. T., Blomqvist, S., Ehnholm, C., Hyvonen, M., Jauhiainen, M., Torstila, I. & Miettinen, T. A. (1993) Sitostanol ester in dietary oil reduces serum cholesterol. Effect on serum plant sterols and cholesterol precursors. J. Lipid Res. 34: 1535–1544.
Vanhanen, H. T., Kajander, J., Lehtovirta, H. & Miettinen, T. A. (1994) Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. Clin. Sci. (Lond.) 87: 61–67.
Weihrauch, J.L. & Gardner, J. M. (1978) Sterol content of foods of plant origin. J. Am. Diet. Assoc. 73: 39–47.
Westrate, J. A. & Meijer, G. W. (1998) Plant sterol-enriched margarines and reduction of plasma total and LDL-cholesterol concentrations in normocholesterolemic and mildly hypercholesterolaemic subjects. Eur. J. Clin. Nutr. 52: 334–343.
Xu, G., Salen, G., Tint, G. S., Batta, A. K. & Shefer, S. (1999) Campestanol (24-methyl-5$\alpha$-cholestan-3$\beta$-ol) absorption and distribution in New Zealand White rabbits: effect of dietary sitostanol. Metabolism 48: 363–368.